

We claim:

- 1 1. A plasmid comprising:
  - 2 a primer sequence capable of annealing to a first portion of nucleic acid
  - 3 encoding a polypeptide;
  - 4 a collar sequence capable of annealing to a second portion of the nucleic acid
  - 5 encoding a polypeptide, the second portion of the nucleic acid encoding a polypeptide
  - 6 being at least 20 nucleotides removed from the first portion of the nucleic acid
  - 7 encoding a polypeptide; and
  - 8 at least one restriction site located between the primer and collar sequences.

1 1 2. A plasmid as in claim 1 wherein the primer and collar sequences are capable of  
2 annealing to first strand cDNA encoding a polypeptide.

1 1 2. 3. A plasmid as in claim 1 wherein the primer and collar sequences are capable of  
2 annealing to mRNA encoding a polypeptide.

1 1 2. 4. A plasmid as in claim 1 wherein the primer and collar sequences are capable of  
2 annealing to mRNA encoding at least a portion of an antibody.

1 1 2. 3. 4. 5. A plasmid as in claim 1 wherein the collar sequence is capable of annealing to  
2 a portion of the nucleic acid encoding a polypeptide that is remote in the 5' direction  
3 from the portion of the nucleic acid to which the primer sequence is capable of  
4 annealing.

1 1 6. A host cell transformed with a plasmid of claim 1.

1 1 7. A method of producing nucleic acid encoding a polypeptide, the method  
2 comprising the steps of :

1       a) providing a plasmid containing a primer sequence adapted to anneal to a  
2 first portion of nucleic acid encoding a polypeptide and a collar sequence adapted to  
3 anneal to a second portion of the nucleic acid encoding a polypeptide, the second  
4 portion of the nucleic acid encoding a polypeptide being at least 20 nucleotides  
5 removed from the first portion of the nucleic acid encoding a polypeptide;  
6           b) cleaving the plasmid at one or more restriction sites, the one or more  
7 restriction sites being located on the plasmid between the primer and collar  
8 sequences; and  
9           c) exposing the cleaved plasmid to nucleic acid encoding a polypeptide in an  
10 aqueous environment containing nucleotides and one or more enzymes selected from  
11 the group consisting of polymerase, reverse transcriptase, ligases and combinations  
12 thereof to form a nucleic acid strand that is complementary to the nucleic acid  
13 encoding a polypeptide, the complementary nucleic acid strand being formed between  
14 the primer and collar sequences whereby the plasmid is ligated and circularized.

1       8.      The method of claim 7 further comprising the step of removing the  
2 complementary nucleic acid strand from the plasmid.

1       9.      A method as in claim 7 wherein the step of cleaving the plasmid comprises  
2 exposing the plasmid to an oligonucleotide that hybridizes to the one or more  
3 restriction sites on the plasmid prior to exposing the plasmid to a restriction enzyme.

1       10.     A method as in claim 7 wherein the step of exposing the cleaved plasmid to a  
2 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to mRNA  
3 encoding a polypeptide.

1       11.     A method as in claim 10 wherein the cleaved plasmid is exposed to mRNA  
2 encoding at least a portion of an antibody.

1    12. A method as in claim 7 wherein the step of exposing the cleaved plasmid to a  
2 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to first  
3 strand cDNA.

1    13. A host cell transformed with a circularized plasmid produced in step c) of claim  
2 7.

1    14. A polypeptide produced using the plasmid of claim 1.

1    15. A polypeptide produced using the circularized plasmid produced in step c) of  
2 claim 7.

1    16. A plasmid comprising:

2              a primer sequence adapted to anneal to a first portion of a nucleic acid  
3 encoding a polypeptide;

4              a collar sequence adapted to anneal to a second portion of the nucleic acid  
5 encoding a polypeptide, the second portion of the nucleic acid encoding a polypeptide  
6 being at least 20 nucleotides removed from the first portion of the nucleic acid  
7 encoding a polypeptide; and

8              a nucleic acid strand that is complementary to the nucleic acid encoding a  
9 polypeptide, the complementary nucleic acid strand being formed in situ between the  
10 primer and collar sequences.

1    17. A plasmid as in claim 16 wherein the primer and collar sequences are adapted  
2 to anneal to first strand cDNA encoding a polypeptide.

1    18. A plasmid as in claim 16 wherein the primer and collar sequences are adapted  
2 to anneal to mRNA encoding a polypeptide.

1    19. A plasmid as in claim 16 wherein the primer and collar sequences are adapted  
2 to anneal to a portion of mRNA encoding at least a portion of an antibody.

- 1 20. A host cell transformed with a plasmid of claim 16.
- 1 21. A polypeptide produced using a plasmid of claim 16.
- 1 22. A plasmid containing a nucleic acid sequence encoding at least a portion of a  
2 polypeptide prepared in accordance with the method of claim 7.
- 1 23. A plasmid comprising:  
2       a downstream primer capable of annealing to a first portion of mRNA encoding  
3 at least a portion of an antibody;  
4       an upstream collar sequence capable of annealing to a second portion of the  
5 mRNA encoding at least a portion of an antibody; and  
6       at least one restriction site located between the downstream primer and  
7 upstream collar sequence.
- 1 24. A plasmid as in claim 23 wherein the upstream collar sequence is capable of  
2 annealing to a portion of the mRNA encoding a framework region of an antibody.
- 1 25. A plasmid as in claim 23 wherein the upstream collar sequence is capable of  
2 annealing to a leader sequence of the mRNA encoding an antibody.
- 1 26. A plasmid as in claim 23 wherein the upstream collar sequence is capable of  
2 annealing to a portion of the mRNA encoding a framework region associated with a  
3 light chain of an antibody.
- 1 27. A plasmid as in claim 23 wherein the upstream collar sequence is capable of  
2 annealing to a portion of the mRNA encoding a framework region associated with a  
3 heavy chain of an antibody.
- 1 28. A plasmid as in claim 23 wherein the downstream primer is capable of  
2 annealing to a portion of the mRNA encoding a constant region of an antibody.

1 29. A plasmid as in claim 23 wherein the downstream primer is capable of  
2 annealing to a portion of the mRNA encoding a constant region associated with a light  
3 chain of an antibody.

1 30. A plasmid as in claim 23 wherein the downstream primer is capable of  
2 annealing to a portion of the mRNA encoding a framework two (FR2), framework three  
3 (FR3) or framework four (FR4) region associated with a light chain of an antibody.

1 31. A plasmid as in claim 23 wherein the downstream primer is capable of  
2 annealing to a portion of the mRNA encoding a constant region associated with a  
3 heavy chain of an antibody.

1 32. A plasmid as in claim 23 wherein the downstream primer is capable of  
2 annealing to a portion of the mRNA encoding a framework two (FR2), framework three  
3 (FR3) or framework four (FR4) region associated with heavy chain of an antibody.

1 33. A plasmid as in claim 23 wherein the downstream primer comprises SEQ. ID.  
2 NO: 4.

1 34. A plasmid as in claim 23 wherein the downstream primer comprises SEQ. ID.  
2 NO: 8.

1 35. A plasmid as in claim 23 wherein the upstream collar sequence comprises  
2 SEQ. ID. NO: 3.

1 36. A plasmid as in claim 23 wherein the upstream collar sequence comprises  
2 SEQ. ID. NO: 7.

1 37. A host cell transformed with a plasmid of claim 23.

- 1 38. A method of producing nucleic acid encoding at least a portion of an antibody,  
2 the method comprising the steps of :
- 3       a) providing a plasmid containing a downstream primer adapted to anneal to a  
4 first portion of mRNA encoding at least a portion of an antibody and an upstream collar  
5 sequence adapted to anneal to a second portion of the mRNA encoding at least a  
6 portion of an antibody;
- 7       b) cleaving a plasmid at one or more restriction sites, the one or more  
8 restriction sites being located on the plasmid between the downstream primer and  
9 upstream collar sequence;
- 10      c) exposing the cleaved plasmid to the mRNA encoding at least a portion of an  
11 antibody in an aqueous environment containing reverse transcriptase and nucleotides  
12 to form a nucleic acid strand that is complementary to the mRNA, the complementary  
13 nucleic acid strand being formed between the downstream primer and upstream collar;  
14 and
- 15      d) ligating the complementary nucleic acid strand to the upstream collar  
16 whereby the plasmid is ligated and circularized.
- 1 39. The method of claim 38 further comprising the step of removing the  
2 complementary nucleic acid strand from the plasmid.
- 1 40. A method as in claim 38 wherein the step of cleaving the plasmid comprises  
2 exposing the plasmid to a restriction enzyme.
- 1 41. A method as in claim 40 wherein the step of cleaving the plasmid comprises  
2 exposing the plasmid to an oligonucleotide that hybridizes to the one or more  
3 restriction sites on the single strand plasmid prior to exposing the plasmid vector to a  
4 restriction enzyme.
- 1 42. A method as in claim 38 wherein the step of exposing the cleaved plasmid to  
2 the mRNA forms a complementary nucleic acid strand encoding at least a light chain  
3 of an antibody.

1 43. A method as in claim 38 wherein the step of exposing the cleaved plasmid to  
2 the mRNA forms a complementary nucleic acid strand encoding at least a heavy chain  
3 of an antibody.

1 44. A method as in claim 39 wherein the step of removing the complementary  
2 nucleic acid strand from the plasmid comprises digesting the plasmid containing the  
3 complementary nucleic acid strand.

1 45. A method as in claim 44 wherein the step of digesting the plasmid containing  
2 the complementary nucleic acid strand comprises the steps of hybridizing a  
3 oligonucleotide to a portion of the single strand antibody DNA to provide a double  
4 stranded restriction site and exposing the double stranded restriction site to a  
5 restriction enzyme.

1 46. A method as in claim 38 wherein the step of providing a plasmid comprises  
2 providing a plasmid wherein the upstream collar sequence is adapted to anneal to a  
3 portion of the mRNA encoding a framework region of an antibody.

1 47. A method as in claim 38 wherein the step of providing a plasmid comprises  
2 providing a plasmid wherein the upstream collar sequence is adapted to anneal to a  
3 portion of the mRNA encoding a framework region associated with a light chain of an  
4 antibody.

1 48. A method as in claim 38 wherein the step of providing a plasmid comprises  
2 providing a plasmid wherein the upstream collar sequence is adapted to anneal to a  
3 portion of the mRNA encoding a framework region associated with a heavy chain of an  
4 antibody.

1 49. A method as in claim 38 wherein the step of providing a plasmid comprises  
2 providing a plasmid wherein the downstream primer is adapted to anneal to a portion  
3 of the mRNA encoding a constant region of an antibody.

1   50. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the downstream primer is adapted to anneal to a portion  
3   of the mRNA encoding a constant region associated with a light chain of an antibody.

1   51. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the downstream primer is adapted to anneal to a portion  
3   of the mRNA encoding a constant region associated with a heavy chain of an  
4   antibody.

1   52. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the upstream collar sequence comprises SEQ. ID. NO: 3.

1   53. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the upstream collar sequence comprises SEQ. ID. NO: 7.

1   54. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the downstream primer comprises SEQ. ID. NO: 4.

1   55. A method as in claim 38 wherein the step of providing a plasmid comprises  
2   providing a plasmid wherein the downstream primer comprises SEQ. ID. NO: 8.

1   56. A host cell transformed with a circularized plasmid produced in step d) of claim  
2   38.

1   57. An antibody or antibody fragment produced using the plasmid of claim 23.

1   58. An antibody or antibody fragment produced using the circularized plasmid  
2   produced in step d) of claim 38.

1   59. A plasmid comprising:

1           a downstream primer adapted to anneal to a first portion of mRNA encoding at  
2 least a portion of an antibody;  
3           an upstream collar sequence adapted to anneal to a second portion of the  
4 mRNA encoding at least a portion of an antibody; and  
5           a nucleic acid strand that is complementary to the mRNA, the complementary  
6 nucleic acid strand being formed in situ between the downstream primer and upstream  
7 collar sequence to close the plasmid.

1 60. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to  
2 anneal to a portion of the mRNA encoding a framework region of an antibody.

1       1 61. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to  
2 anneal to a portion of the mRNA encoding a framework region associated with a light  
3 chain of an antibody.

1       1 62. A plasmid as in claim 59 wherein the upstream collar sequence is adapted to  
2 anneal to a portion of the mRNA encoding a framework region associated with a heavy  
3 chain of an antibody.

1       1 63. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal  
2 to a portion of the mRNA encoding a constant region of an antibody.

1 64. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal  
2 to a portion of the mRNA encoding a constant region associated with a light chain of  
3 an antibody.

1 65. A plasmid as in claim 59 wherein the downstream primer is adapted to anneal  
2 to a portion of the mRNA encoding a constant region associated with a heavy chain of  
3 an antibody.

1 66. A plasmid as in claim 59 wherein the upstream collar sequence comprises  
2 SEQ. ID. NO: 3.

1 67. A plasmid as in claim 59 wherein the upstream collar sequence comprises  
2 SEQ. ID. NO: 7.

1 68. A plasmid as in claim 59 wherein the downstream primer comprises SEQ. ID.  
2 NO: 4.

1 69. A plasmid as in claim 59 wherein the downstream primer comprises SEQ. ID.  
2 NO: 8.

1 70. A host cell transformed with a plasmid of claim 59.

1 71. An antibody or antibody fragment produced using the plasmid of claim 59.

1 72. A plasmid containing a nucleic acid sequence encoding at least a portion of an  
2 antibody prepared in accordance with the method of claim 38.

1 73. A plasmid as in claim 1 wherein two restriction sites that are the same or  
2 different are located between the downstream primer and upstream collar sequences.

1 74. A plasmid as in claim 23 wherein two restriction sites that are the same or  
2 different are located between the downstream primer and upstream collar sequence.

1 75. A library containing a repertoire of antibodies or antibody fragments made using  
2 the plasmid of claim 23.

1 76. A library containing a repertoire of antibodies or antibody fragments made using  
2 the method of claim 38.

1 77. A library containing a repertoire of antibodies or antibody fragments made using  
2 the plasmid of claim 59.

1 78. A method of producing nucleic acid encoding a polypeptide, the method  
2 comprising the steps of :  
3       a) providing a plasmid containing a primer sequence adapted to anneal to a  
4 first portion of nucleic acid encoding a polypeptide;  
5       b) cleaving the plasmid at one or more restriction sites, the one or more  
6 restriction sites being located on the plasmid to provide a cleaved plasmid having the  
7 primer at one end thereof and a free end; and  
8       c) exposing the cleaved plasmid to nucleic acid encoding a polypeptide in an  
9 aqueous environment containing nucleotides and one or more enzymes selected from  
10 the group consisting of polymerase, reverse transcriptase, ligases and combinations  
11 thereof to form a nucleic acid strand that is complementary to the nucleic acid  
12 encoding a polypeptide, the complementary nucleic acid strand being formed attached  
13 to the primer and having a free end.

1 79. A method as in claim 78 further comprising the step of joining the free end of  
2 the complementary nucleic acid strand to the free end of the plasmid whereby the  
3 plasmid is circularized.

1 80. A method as in claim 79 wherein the step of joining comprises providing a  
2 bridging oligonucleotide.

1 81. The method of claim 78 further comprising the step of removing the  
2 complementary nucleic acid strand from the cleaved plasmid.

1 82. A method as in claim 78 wherein the step of exposing the cleaved plasmid to a  
2 nucleic acid encoding a polypeptide comprises exposing the cleaved plasmid to first  
3 strand cDNA.

- 1 83. A method as in claim 82 wherein the cleaved plasmid is exposed to antibody
  - 2 cDNA encoding at least a portion of an antibody.  
  - 1 84. A method as in claim 82 wherein the first strand cDNA is cleaved at a restriction
  - 2 site prior to being exposed to the cleaved plasmid.

— 18 —

卷之三